Identification and characterization of genes encoding multicopper-oxidase enzymes from *Rhodococcus opacus* R7 for polyethylene degradation

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Polyethylene is the most abundant petroleum-based plastic material produced globally, and one of the most resistant to biodegradation, resulting in massive accumulation in the environment.

Among few strains showing the ability to grow in the presence of polyethylene as the sole carbon and energy source, *Rhodococcus opacus* R7 can degrade this plastic polymer in a short range of time without any physical or chemical pretreatments. Based on previous RNA-seq analysis performed after R7 growth on PE, three genes putatively involved in the first step of PE oxidation were identified (*LMCO1*, *LMCO2*, and *LMCO3* genes). The comparison of R7 *LMCO* genes to reference laccase-like enzyme sequences showed high similarity. In particular, LMCO gene product identified in *R. ruber* C208 - able to grow on PE - showed an amino acidic identity of 48%, 53%, and 23% with respect to R7 LMCO1, LMCO2, and LMCO3, respectively.

Besides, enzymatic assays on the supernatant of wild type R7 collected at different times of growth in the presence of PE confirmed the activation of genes encoding for multicopper oxidases - laccase-like enzymes confirming the activation of superficial or releasing extracellular enzymes.

On this basis, R7 LMCO genes were isolated and characterized. They were amplified by PCR and subsequently cloned in an *E. coli-Rhodococcus* shuttle vector, pTipQC2, to prove their expression in a heterologous strain. Since *E. coli* does not efficiently express *Rhodococcus* genes, the genes were transferred in *Rhodococcus erythropolis* strain AP. Their expression levels were assessed by a laccase enzymatic assay in the presence of the supernatant or cell extract of the recombinant strains, and 2,6-dimethoxyphenol as substrate. The preliminary outcomes showed that these cloned genes belong to laccase family, indeed they showed high level of laccase activity after induction of the recombinant strain.

These data provided important knowledge on LMCO systems and experiments to increase enzyme activity in both wild type and recombinant strain will be fundamental to better elucidate their functionality.